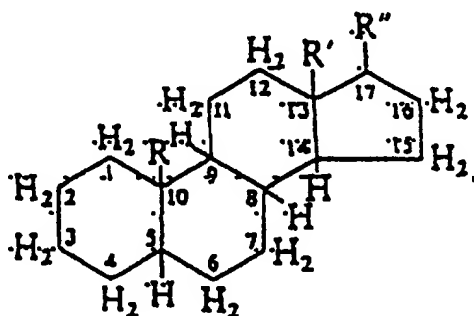


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(54) Title: NITRATE ESTERS OF CORTICOID COMPOUNDS AND PHARMACEUTICAL APPLICATIONS THEREOF

(1)

(57) Abstract

Compounds of the general formula B-X₁-NO₂ or their esters or salts, where B has structure (1) where there may be substituents in place of the hydrogens H in the CH group or two hydrogens H₂ in the CH₂ group shown in the general formula; R and R' are equal or different one from the other and may be hydrogen or linear or branched alkyls having from 1 to 4 carbon atoms, preferably R = R' = CH₃; B being a corticosteroid residue; R'' is -(CO-L)_t-(X)_{t1}- where t and t1 are integers equal or different one from the other and equal to 0 or 1; where L is a bivalent connecting group; X is equal to X₀ where X₀ = 0, NH, NR_{1C} where R_{1C} is a linear or branched alkyl having from 1 to 10 C atoms; or equal to X₂ where X₂ is equal to OH, CH₃, Cl, N(-CH₂-CH₃)₂, SCH₂F, SH; X₁ is a bivalent connecting bridge YO where Y is a C₁-C₂₀ alkylene.

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NITRATE ESTERS OF CORTICOID COMPOUNDS AND PHARMACEUTICAL APPLICATIONS THEREOF

The present invention relates to preparation of new corticoid compounds.

In particular it relates to steroid-structured compounds having anti-inflammatory, immunodepressive and angiostatic activities (the so-called steroid anti-inflammatory drugs).

The compounds according to the present invention are therapeutically useful in the treatment of pathologic conditions where generally corticosteroid (corticoids) preparations are used, but with increased benefits.

This represents an unexpected advantage over the known corticoids products. In fact, by taking into account the various defined therapeutic uses of a specific product, it is always possible, with the new products of the present invention, to find a better combination of results with respect to the known corticoids. Contrary to any expectation the products of the present invention are characterised by the fact that they show an improved therapeutic profile: high activity combined with low side-effects.

Corticoids are well known as a first-choice pharmacological measure in the treatment of inflammatory disease. Drugs in this category - which include, for example, hydrocortisone, cortisone, prednisolone, prednisone, fludrocortisone,

tisone, desoxycorticosterone, methylprednisolone, triamcinolone, paramethasone, betametasone, dexamethasone, triamcinolone acetonide, fluocinolone acetonide, beclomethasone, acetoxypregnelone, etc. - have marked pharmacotoxicological effects on various organs. Because of this, the clinical use and discontinued use thereof cause a series of side effects, some of which are very severe. See for example Goodman & Gilman: "The Pharmaceutical Basis of Therapeutics", 9th Ed., pages 1459-1465, 1996.

These toxic effects include:

- those on bone which lead to changed cell metabolism and a high frequency of osteoporosis;
- those on the cardiovascular system which cause hypertensive reactions;
- those on the gastrointestinal tract which cause gastric damage.

See for instance Martindale: "The Extrapharmacopoeia", 30th Ed., pages 712-723, 1993.

According to the above mentioned art it appears to be almost impossible for therapeutic activities to be separated from side effects, see Goodman et al., as mentioned above, at page 1474.

Known in the art are non-steroid anti-inflammatory

drugs either with or without acidic ending, see patents WO 94/04484, WO 94/12463, WO 95/09831, WO 95/30641 for non-acidic ending and the patents therein mentioned for those with acidic ending.

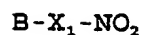
However, it should be noted that steroid compounds are completely different from non-steroid compounds chemically, pharmacologically and biochemically as the pharmacotoxicological mechanism of action of non-steroid products is based on inhibition of one or more cyclo-oxygenases (COX), while steroid products have nothing to share with COX and have more complex pharmacotoxicological mechanisms of action which have not yet been fully explained.

It is well known that these two groups of compounds are listed in completely separate categories in international pharmacopoeias.

The applicant has surprisingly and unexpectedly found corticosteroids (corticoids) which are very effective, even superior to those in the known art, and have, at the same time, a higher tolerance than the known corticoids as unexpectedly they do not cause the above side effects, or when they do, these are lower.

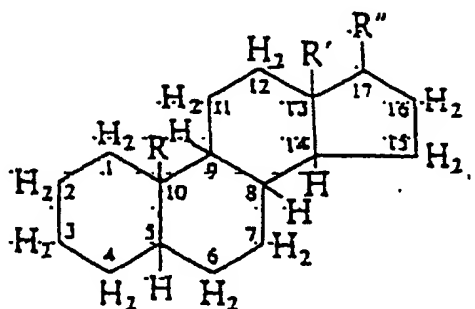
An object of the present invention are corticosteroids and their use as anti-inflammatory, immunosuppressive and

angiostatic agents having the general formula:



or their esters or salts, where:

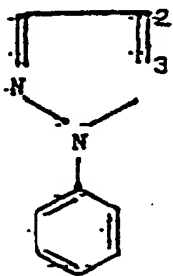
B has the following structure:



where, in place of the hydrogens H in the CH group or two hydrogens H₂ in the CH₂ group shown in the general formula, there may be the following substituents:

at position 1-2: there may be a double bond;

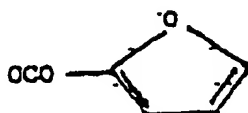
at position 2-3: there may be the following substituent:



at position 2: there may be Cl, Br;

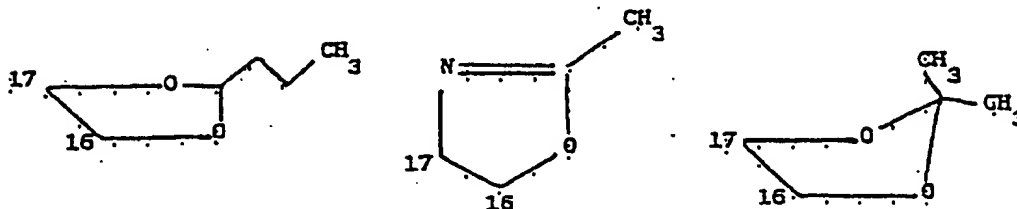
at position 3: there may be CO, -O-CH₂-CH₂-Cl, OH;

at position 4-5: there may be a double bond;
 at position 5-6: there may be a double bond;
 at position 6: there may be Cl, F, CH₃, -CHO;
 at position 7: there may be Cl;
 at position 9: there may be Cl, F;
 at position 11: there may be OH, CO, Cl;
 at position 16, there may be CH₃, OH, =CH₂;
 at position 17: there may be OH, CH₃, OCO(O)_{ua}(CH₂)_{va}CH₃, or



where ua is an integer equal to 0 or 1, va is an integer from 0 to 4;

at positions 16-17: there may be the following groups

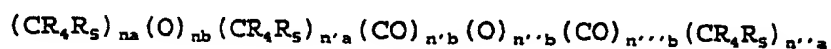


R and R' are equal or different one from the other and may be hydrogen or linear or branched alkyls having from 1 to 4 carbon atoms, preferably R = R' = CH₃;
 B being a corticosteroid residue;

R'' is $-(CO-L)_t-(X)_{t1}-$

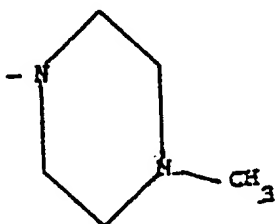
where t and $t1$ are integers equal or different one from the other and equal to 0 or 1, provided that they cannot be both equal to 0 when B contains no -OH groups;

the bivalent bridging group L is selected from:



where na , $n'a$ and $n''a$ are equal or different one from the other and are integers from 0 to 6, preferably from 1 to 3; nb , nb' , $n''b$ and $n'''b$ are equal or different one from the other and are integers equal to 0 or 1; R_4 and R_5 are equal or different one from the other and are chosen from H, linear or branched alkyl having from 1 to 5 carbon atoms, preferably from 1 to 3;

X is equal to $X_0 = 0$, NH, NR_{1c} where R_{1c} is a linear or branched alkyl having from 1 to 10 C atoms; or equal to X_2 where X_2 is equal to OH, CH_3 , Cl, $N(-CH_2-CH_3)_2$, SCH_2F , SH,

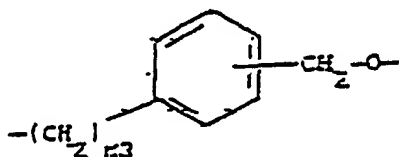


X_1 is a bivalent connecting bridge chosen from:

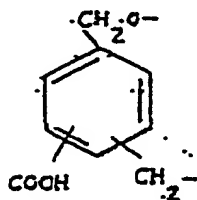
- YO

where Y is a linear or whenever possible branched C₁-C₂₀ alkylene, preferably having from 2 to 5 carbon atoms, or an optionally substituted cycloalkylene having from 5 to 7 carbon atoms;

- Y₁ selected from



where n₃ is an integer from 0 to 3;



- $-(\text{CH}_2-\underset{\text{ONO}_2}{\text{CH}}-\text{CH}_2-\text{O})_{\text{nf}'}-$

where nf' is an integer from 1 to 6, preferably from 2 to 4;

- $-(\underset{\text{R}_{1f}}{\text{CH}}-\text{CH}_2-\text{O})_{\text{nf}}-$

where R_{1f} = H, CH₃, and nf is an integer from 1 to 6, preferably from 2 to 4.

The compounds which can be mentioned, and which are those preferred, are the ones listed below where B can be obtained according to the known processes of the art.

For example, the precursors and related processes described for example in The Merck Index, 12th Ed. of 1996, herein incorporated by reference, can be mentioned as precursors and related processes. The precursors (according to the Merck nomenclature) include the following, where H₂, H, R, R', R'' have the meaning as defined in the compounds listed below: budesonide, hydrocortisone, alclometasone, algestone, beclomethasone, betamethasone, chloroprednisone, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, corticosterone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difluprednate, fluazacort, flucloronide, flumethasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortyn butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, formocortol, halcinonide, halobetasol propionate, halometasone, halopredone acetate, hydrocortamate, loteprednol etabonate, medrysone, meprednisone, methylprednisolone, mometasone furoate, parametasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone,

prednival, prednylidene, rimexolone, triamcinolone, triamcinolone acetonide, 21-acetoxypregnenolone, cortivazol, amcinonide, fluticasone proprionate, mazipredone, tixocortol, triamcinolone hexacetonide.

The X_1 connecting bridges as above defined are obtainable by using the methods known in the art as indicated above or by modifying the known methods by introducing X_1 bridges when these are different from the connecting bridges described in the listed patents, using processes known in the art. Generally the connection between B and X_1 is, as seen, of an ester or amide type (NH or NR_{10} , as defined in X). Any well known synthetic route for forming these bonds can be used to form this connection.

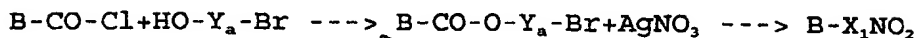
In the case of esters, the most direct synthethic route includes:

reaction of acyl chlorides $B-CO-Cl$ in halogen alcohols of the $HO-Y_a-Cl$, $HO-Y_a-Br$, $HO-Y_a-I$ type, where Y_a is equal to Y or Y_1 without the oxygen atom, in test conditions which are part of the known art.

The reaction products of formula $B-CO-O-Y-Cl(Br, I)$ can also be obtained by reacting the sodium or potassium salts of salts acids $B-CO-OH$ with dihalogen derivatives of the general formula Y_aCl_2 , Y_aBr_a or Y_aI_2 , ClY_aBr , ClY_aI , BrY_aI .

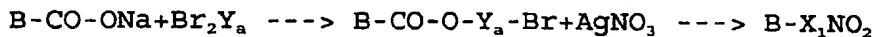
The reaction products are converted into the final products by reacting with AgNO_3 in acetonitrile according to what is known in the literature.

The general scheme is as follows:



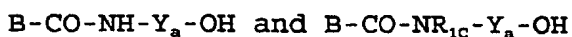
where $\text{X}_1 = \text{Y}_a\text{O}$.

The general scheme may also be as follows:



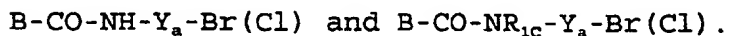
where $\text{X}_1 = \text{Y}_a\text{O}$.

In this case of amide, the synthetic sequence includes reaction of the same acyl chlorides BCOCl with aminoalcohols of the general formula $\text{NH}_2\text{-Y}_a\text{-OH}$, $\text{NHR}_{1c}\text{-Y}_a\text{-OH}$ to give amides of the general formula:



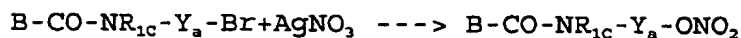
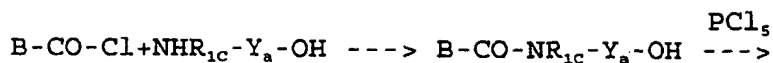
according to known methods.

Reaction of these amides with halogenating agents such as, for example PCl_5 , PBr_3 , SOCl_2 , etc., gives the halogen derivatives of the general formula:



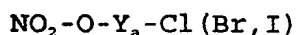
The latter give the final products BX_1NO_2 by reacting with AgNO_3 in acetonitrile according to methods known in the literature.

The sequence may be represented as:



where Y_aO is X_1 .

An alternative route to ester formation is reaction of the sodium or potassium salts of the acids with the nitric esters of halogen alcohols of the general formula:



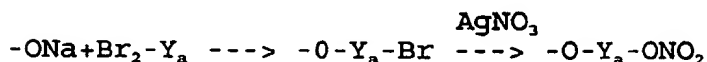
to directly give the products of the invention.

The reaction scheme is as follows:



where Y_aO is X_1 .

Other synthetic routes similar to those described above include those in which the dihalogen derivative Br_2Y_a is reacted with enolates. The reaction products are then converted by reacting with AgNO_3 in acetonitrile according to the above reaction. The general scheme is shown for an -OH in group B, of the type $-\text{CH}_2\text{-OH}$, $=\text{CH-OH}$, is as follows:



The processes to obtain these X_1 connecting groups are described in patent application WO95/30641 herein incorporated by reference.

As said above the compounds of the invention of

formula B-X₁-NO₂, or their pharmaceutical compositions, are used for the treatment of diseases in which the well known corticoids products are employed.

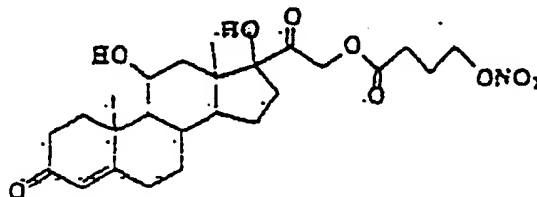
In particular, it can be specifically mentioned the use in respiratory disorders, e.g. antiasthmatic, the use as antiarthritic, antipruritic, antipsoriatic, antieczematic; the use in vascular disorders, e.g. as angiostatic, the use in immunology disorders, e.g. as immunosuppressive.

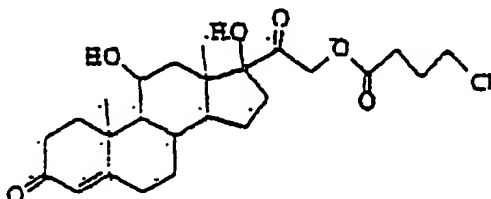
The compounds, or their compositions, of the present invention can be administered for example by oral, rectal (intestinal disorders), parenteral route or by local (dermal, topical, transdermal, ocular, inhalatory, etc.) application.

The following examples are given only for illustrative purpose as an explanation but not as a limitation of the present invention.

EXAMPLE 1

CHEMICAL SYNTHESIS: preparation of hydrocortisone nitroderivative (HCN)



EXAMPLE 1APreparation of hydrocortisone (4-chloro)butanoate

4 portions of 4-chlorobutanoylchloride (0.32 ml x 4) and triethylamine (0.3 g x 4) were added in 24 hours to a solution of hydrocortisone (1 g) in CHCl_3 , dried over P_2O_5 , and stirred for 3 days. The solution was treated with water, the organic phase was separated, dried (Na_2SO_4) and deprived of the solvent at reduced pressure. The crude residue was ground with hexane and CH_2Cl_2 to give a white solid with a 53% yield by weight, which had a melting point (m.p.) of 155°C .

The product was characterised by mass spectrometry: M^+493 .

^1H NMR (300 MHz CDCl_3): 0.95 (3H, s, CH_3), 1.45 (3H, s, CH_3), 2.12 (2H, t, CH_2 in 2), 2.6 (2H, t, CH_2COO), 3.65 (2H, t, CH_2Cl), 4.45 (1H, m, CHOH), 4.35 and 5.05 (2H, 2d, COCH_2O), 5.70 (1H, s, olefin H).

Preparation of hydrocortisone (4-nitroxy)butanoate

AgNO_3 (0.2 g) was added to a solution of hydrocortisone-

4-chlorobutanoate prepared as above (0.23 g) in acetonitrile (70 ml) and refluxed for 16 hours. The solution was deprived of the solvent at reduced pressure and chromatographed on silica gel using a solution of ethyl acetate and CH_2Cl_2 (3:7) as an eluant.

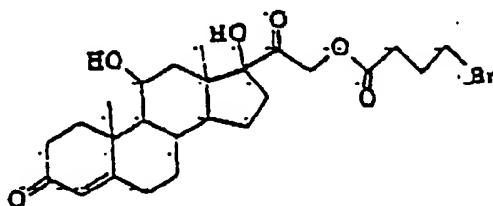
Cortisone 4-nitroxybutanoate was recovered from the head fractions.

The product was characterised by ^1H NMR (300 MHz CDCl_3): 0.95 (3H, s, CH_3), 1.45 (3H, s, CH_3), 2.12 (2H, t, CH_2 in 2), 2.6 (2H, t, CH_2COO), 4.45 (1H, m, CHOH), 4.45 (2H, t, $\text{CH}_2\text{O-NO}_2$), 4.35 and 5.05 (2H, 2d, COCH_2O), 5.68 (1H, s, olefin H).

EXAMPLE 1B

The product from Example 1A was also prepared using another synthetic route.

Preparation of hydrocortisone 4-bromobutanoate



Five portions of 4-bromobutanoylchloride (0.35 ml x 5) and potassium carbonate (0.4 g x 5) were added in 24 hours to a solution of hydrocortisone (1 g) in CHCl_3 dried over P_2O_5 and stirred for 5 days. The solution was treated with water,

the organic phase was separated, dried (Na_2SO_4) and deprived of the solvent at reduced pressure.

Preparation of hydrocortisone 4-nitroxybutanoate (HCN)

AgNO_3 (0.2 g) was added to a solution of hydrocortisone 4-bromobutanoate prepared as above (0.23 g) in acetonitrile (70 ml) and stirred for 48 hours at room temperature.

The solution was deprived of the solvent at reduced pressure and chromatographed on silica gel using a solution of ethyl acetate and CH_2Cl_2 (3:7) as an eluant.

Cortisone 4-nitroxybutanoate was recovered from the head fractions and characterised by mass spectrometry: M^+ 493. The spectrum was the same as that shown in Example 1A.

EXAMPLE 2

Evaluation of safety and activity

The products were administered in a 2%-by-weight carboxymethyl cellulose suspension during in vivo tests, while a 0.1%-by-weight dimethylsulphoxide suspension was used for in vitro studies.

The test groups always included 8 samples (except when differently stated in the examples) for adequate statistical evaluation, to be carried out when necessary according to common statistical procedures.

EXAMPLES 2A**Acute toxicity study**

The acute toxicity of the product from Example 1A was roughly evaluated by orally administering a single dose of substance to a group of 10 mice of the Swiss strain.

Death incidence and appearance of toxic symptoms were observed during a period of 14 days after the compound administration.

The animals showed no sign of apparent toxicity even after administration of a 50 mg/kg dose.

EXAMPLE 2B**Study of antiarthritic activity**

Adjuvant arthritis was induced in male rats of Lewis strain, weighing 170 ± 15 g by intracaudal injection of 0.6 mg of *Mycobacterium butyricum* (Difco) suspended in 0.1 ml of mineral oil. The animals were treated with a vehicle made up of an intraperitoneal (i.p.) 2%-by-weight suspension of carboxymethyl cellulose in water, with intraperitoneal hydrocortisone of HCN (a suspension as described above) at doses of 5 mg/kg or doses of 10 mg/kg, starting from the first day after mycobacterium inoculation.

Arthritis development was assessed 21 days later. To the arthritic lesions an arbitrary score was assigned accor-

ding to the following scale:

- hind limbs: 0 to 7 for each (0 for no lesions and 7 for most severe lesions);
- forelimbs: 0 to 4.5 for each (0 for no lesions and 4.5 for most severe lesions);
- tail: 0 to 5 (0 for no lesions and 5 for most severe lesions);
- ears: 0 to 2 for each (0 for no lesions and 2 for most severe lesions);
- nose and eyes: 0 to 1 ofr each (0 for no lesions and 1 for most severe lesions).

The results were expressed as a percentage of inhibition compared to the value obtained in the control group (animals treated withe the vehicle alone).

The result are shown in Table 1.

TABLE 1 - STUDY OF ANTIARTHRITIC ACTIVITY OF COMPOUND HCN VERSUS HYDROCORTISONE IN RATS

COMPOUND	DOSE (mg/kg)	ANTIARTHRITIC ACTIVITY (%)
HYDROCORTISONE	5	40
HYDROCORTISONE	10	55
HCN	5	45
HCN	10	62

As shown by the results in Table 1, the test products were capable of similarly inhibiting development of the arthritic process caused by mycobacterium. However, being the tolerability of HCN much higher than that of hydrocortisone (see ex 2C below), the results in terms of activity are much better in the case of HCN (see for comparison 40% of antiarthritic activity obtained with 5 mg/kg hydrocortisone with respect to 62% obtained with 10 mg/kg HCN).

EXAMPLE 2C

Study of gastric tolerability (safety)

Male Sprague-Dawley rats fasted for 24 hours were treated with 5 to 10 mg/kg of intraperitoneal hydrocortisone or HCN.

Twenty-four hours later the animals were sacrificed, the stomach was removed and tissue was grossly examined for the presence of lesions as described by Del Soldato et al.: "The influence of fasting and cimetidine on the relationship between ulcerogenic and anti-inflammatory properties of cimetidine", Br.J.Pharmacol. 67, 33-37, 1979. The degree of severity of the disease was evaluated according to common methods and expressed as arbitrary values. The results are shown in Table 2.

TABLE 2 - STUDY OF GASTRIC TOLERABILITY OF COMPOUND HCN
VERSUS HYDROCORTISONE IN RATS

COMPOUND	DOSE (mg/kg)	GASTRIC TOLERABILITY
HYDROCORTISONE	5	2.0
HYDROCORTISONE	10	3.5
HCN	5	0.5*
HCN	10	1.2*

Data are expressed as arbitrary values according to the following scale: 0 = absent; 1 = mild lesions; 2 = moderate lesions; 3 = punctiform ulcers; 4 = severe and numerous ulcers.

* $P < 0.05$ (where P is probability) compared to corresponding value in group treated with hydrocortisone.

As shown in Table 2, the rats treated with hydrocortisone exhibited a marked disease in the gastrointestinal tract, varying in severity from mucosal erosion to ulcer involving the muscle layer, wall adhesions, ascites, peritonitis. In the other groups treated with the vehicle alone or HCN, the damage was much lower or even absent.

EXAMPLE 2D

Study of nitroxysynthetase activity

The nitroxy-synthetase inhibiting activity induced by lipopolysaccharide (LPS) was determined in rat neutrophils

and stomach after administration of one of the test compounds and compared with that obtained after treatment with the suspending vehicle only. Wistar rats fasted for 24 hours before treatment received one of the test compounds intraperitoneally (10 mg/kg) or LPS intravenously (caudal vein) (5 mg/kg). Four hours later the animals were sacrificed. Blood for neutrophil isolation and stomach were removed.

Enzymatic activity was determined according to the method described by Assreuy et al.: "Feedback inhibition of nitric oxide synthase activity by nitric oxide", Br.J.Pharmacol. 108, 833-7, 1993. The results are shown in Table 3.

TABLE 3 - STUDY OF NITROXYSYNTHETASE ACTIVITY IN COMPOUND HCN VERSUS HYDROCORTISONE IN RATS

COMPOUND	DOSE (mg/kg/i.p.)	NITROXYSYNTHETASE ACTIVITY ^a
VEHICLE	-	100
HYDROCORTISONE	10	55*
HCN	10	62*

^a per cent inhibition compared to group treated with vehicle alone

* p < 0.05 compared to corresponding value in group treated with vehicle.

As shown by Table 3, both test products proved to be very effective in inhibiting nitroxysynthetase compared to

the group treated with the vehicle alone.

EXAMPLE 2E

Study of bone toxicity

Bone tissues (parietal bone from rat foetus) grown in vitro according to the method described by Doherty et al. ("The effect of glucocorticoids on osteoblast function. The effect of corticosterone on osteoblast, expression of beta-I integrins", Journal of Bone and Joint Surgery, Series A77/3, 396-404, 1995) was used. Hydrocortisone or HCN or the vehicle were incubated at concentrations of 100 nmol.

Ninety six hours later calcium content and bone dry weight were measured.

The results are shown in Table 4.

TABLE 4 - EFFECT OF HCN AND HYDROCORTISONE ON BONE GROWTH IN RATS

TREATMENT	(nmol)	Calcium $\Delta^{\circ}\%$	DRY TISSUE WEIGHT $\Delta^{\circ}\%$
VEHICLE	-	310	160
HYDROCORTISONE	10	70*	95*
HCN	10	287	149

° Compared to initial value (incubation time zero)

* P < 0.05 compared to values obtained in control group (vehicle)

As shown in Table 4, a significant increase in tissue dry weight and increased calcium were observed after incuba-

tion with the vehicle or HCN. After incubation with hydrocortisone, the calcium content decreased and the bone dry weight did not increase. This shows that this treatment with hydrocortisone adversely affected bone growth.

EXAMPLE 2F

Study of some cardiovascular parameters

The effect of the test products on some cardiovascular parameters was studied in conscious Long Evans rats (350 to 450 g) which were appropriately monitored, as described by Gardiner et al.: "Influence of dexamethasone on the regional haemodynamic responses to lipopolysaccharide in conscious rats: effect of the non-selective endothelin antagonist: SB 209670", Br.J. Pharmacology 117, 49P, 1996. The animals were treated with the vehicle (physiologic saline solution, 0,9% sodium chloride, s.c.) subcutaneous hydrocortisone or HCN (10 mg/kg). Heart rate and blood pressure were recorded 4 hours after treatment.

Table 5 shows the data obtained as per-cent variation from control values.

TABLE 5 - STUDY OF COMPOUND HCN VERSUS HYDROCORTISONE IN SOME CARDIOVASCULAR PARAMETERS IN RATS

COMPOUND	DOSE (mg/kg)	HEART RATE ^a (%)	BLOOD PRESSURE ^b
VEHICLE	-	100	160
HYDROCORTISONE	10	89 ^a	115 ^a
HCN	10	98	103

* $P < 0.05$ compared to group treated with vehicle

^a per-cent change compared to value recorded in group treated with vehicle alone (324±7 beats per minute)

^b per-cent change compared to value recorded in group treated with vehicle alone (101±2 mm Hg)

The results in Table 5 show that the product of the invention HCN does not affect the cardiovascular parameters measured. On the contrary, hydrocortisone used in the known art shows significant pressure as well as cardiac changes.

EXAMPLE 2G

Study of angiostatic activity in rats

Male Wistar rats weighing 180 to 200 g were used according to the procedure described by Andrade et al.: "Quantitative in vivo studies on angiogenesis in a rat sponge model", Brit. J. Exp. Pathol. 68, 755-766, 1987. The neovascularisation was evaluated in relation to blood flow by implanting a small sponge in the subcutaneous tissue for 14 days and determining ¹³³Xe clearance. Briefly, an amount of ¹³³Xe equal to 10µl was injected into the sponge using a small polyethylene cannula. The residual radioactivity from

implantation using a gamma ray detector and the ^{133}Xe clearance for 6 minutes was measured as a percentage of the initial value. The validity of this method for measuring neovascularisation was recently demonstrated by HU et al.: "Correlation of ^{133}Xe clearance, blood flow and histology in the rat sponge model for angiogenesis. Further studies with angiogenic modifiers", Lab. Invest. 72, 601-610, 1995.

The test compounds were administered by the subcutaneous route at a dose of 10 mg/kg from day 1 to day 13 after implantation. ^{133}Xe was measured at day 14 from subcutaneous implantation, the animals were then sacrificed and the weights of thymus and spleen were recorded.

Table 6 shows the data obtained regarding the effect of the test products on neovascularisation and on the weight of spleen and thymus.

TABLE 6 - EFFECT OF HCN AND HYDROCORTISONE ON ^{133}Xe CLEARANCE AND WEIGHT OF SPLEEN AND THYMUS AT DAY 14

TREATMENT	^{133}Xe (%)	SPLEEN (mg)	THYMUS (mg)
VEHICLE	42	663±25	313±28
HYDROCORTISONE	33	642±32	185±17*
HCN	22*	673±38	297±31

* P < 0.05 compared to values obtained in control group (vehicle)

As evident, HCN proved to be capable of exerting a

marked angiostatic effect without changing the weight of spleen or thymus, differently from the reference product.

As it is clear from the whole of the data shown in Tables 1 to 6, the pharmacodynamic activity - anti-arthritic, immunosuppressive and antiangiogenic activities - and tolerability of the nitroderivative are superior than those of the corticoid from the known art.

EXAMPLE 3

Dexamethasone 21-(4-bromobutyrate) [II]

Dexamethasone [I]	3.5 g	8.9 mmol
4-Bromobutyryl chloride	4.06 ml	35 mmol
Potassium carbonate	4.9 g	35 mmol
Tetrahydrofuran	70ml	

The solution of compound I in tetrahydrofuran is portionwise treated with 4-bromobutyryl chloride (0.81 ml x 5) and potassium carbonate (0.98 g x 5) during 7 hours. The mixture is stirred overnight, the solvent is evaporated under vacuum and the residue is treated with ethyl ether and water. The organic layer is separated, washed with water and dried with anhydrous sodium sulfate. After evaporation of the solvent, the residue is purified by silica gel flash column chromatography eluting with t.butyl methyl ether - hexane 1-1 to give:

- less polar compound 1.0 g;
- derivative II 1.5 g (m.p. 184-187 °C; yield 31%)

TLC: t.butyl methyl ether-hexane 2-1.

Dexamethasone 21-(4-nitrooxybutyrate) [III] (compound DXN)

Compound II	1.5 g	2.7 mmol
Silver nitrate	2.4 g	14.1 mmol
Acetonitrile	250	ml

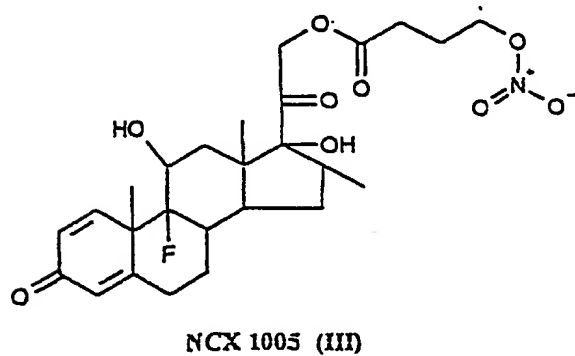
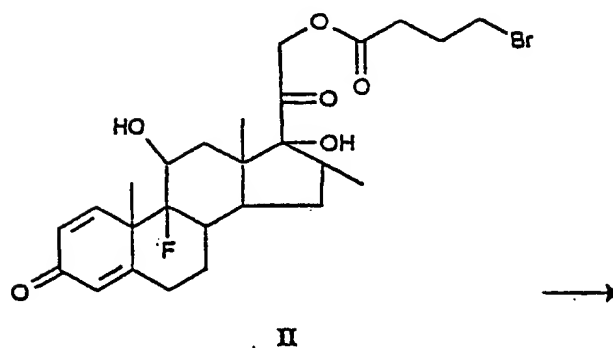
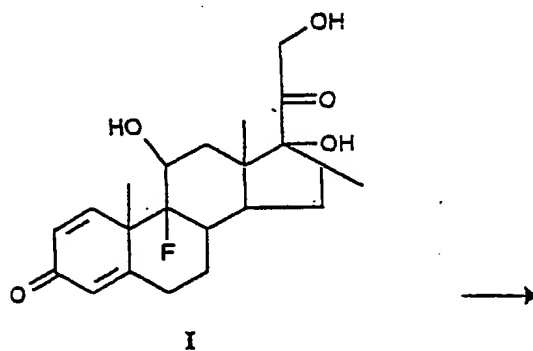
The mixture of compound II and silver nitrate in acetonitrile is refluxed for 7 hours. After filtration of inorganic salts, the solvent is evaporated under vacuum and the residue is treated with ethyl ether. The organic layer is twice washed with water, dried with anhydrous sodium sulfate and evaporated under vacuum. The residue is poured into ethyl ether and filtered to give 1.27 g of pure compound III as a white solid (m.p. 183-185° C; yield 90%)

TLC: t.butyl methyl ether-hexane 2-1.

The following forms are enclosed:

- synthetic scheme;
- NCX 1005 batch 1;
- NCX 1005/1 analysis.

SYNTHETIC SCHEME FOR THE PREPARATION
OF DEXAMETHASONE 21-(4-NITROOXIBUTYRATE)



EXAMPLE 4**Study of the activity on leucocyte accumulation**

Male Swiss albino mice (27-33 g) maintained on a standard chow pellet diet and tap water ad libitum were used. The experiment was done as previously described by Perretti et al. (Perretti M., Solito E., Parente L., "Evidence that endogenous interleukin-1 is involved in neutrophil migration in acute experimental inflammation in rats and mice", Agents Actions, 35,71,1992). Animals were pretreated with zymosan (1mg/0.5ml) i.p. at time 0. Two hours later DEXAMETHASONE (1 mg/kg) (Ex3-I), DXN (Ex3-III) (1mg/kg) or phosphate buffered saline (PBS) was given intravenously. The animals were sacrificed at 4 and 24h the lavage fluids were collected and differential cell counts were performed following staining in Turk's.

Table 7 reports results obtained on the inhibitory effect of the tested compounds on zymosan-induced leucocyte migration in mice. As can be observed the nitroderivative steroid is much more active than dexamethasone.

TABLE 7-Inhibition of neutrophil and monocyte recruitment by DEXAMETHASONE and DXN (1mg/kg) given 2h i.v. after zymosan (1mg/0.5ml) i.p.

treatment	PMN $\times 10^6$ /mouse (time 4h)	% reduction	mono-mØ $\times 10^6$ /mouse (time 24h)	%reduction
vehicle	10.1 \pm 1.0	8.8 \pm 1.3
DXN	4.5 \pm 0.3	55.4	4.5 \pm 0.6	48.8
DEXAMETHASONE	6.4 \pm 0.4	36.6	6.3 \pm 0.2	28.4

EXAMPLE 5**Study of the anti-proliferative activity in human airway
smooth muscle cells**

Human airway smooth muscle cells were cultured by standard explant methods. Tissues were collected into sterile pots containing PBS and penicillin and streptomycin. Under sterile tissue culture conditions, tissues were cut into small pieces (approximately 1 mg weight) and placed into standard medium containing 20% fetal calf serum (FCS) for several days (medium changed every 2-4 days). ^3H -thymidine was measured in the DNA fraction of cells cultured into 48 well plates. Cells were cultured to confluence in the medium containing 10% FCS. Cells were deprived of serum for 24h before the addition of 10% FCS, together with different concentration of steroids. After 24h, ^3H -thymidine was added to the cells for 4h. Cells were washed with phosphate buffered saline and ethanol. The DNA was extracted with sodium hydroxide solution and the ^3H material counted by scintillation. The data represent observations made in triplicate

wells from smooth muscle cultured from one healthy lung donor. Table 8 reports results obtained on the inhibitory effect of the tested compounds on human airway smooth cell proliferation. As can be observed the nitroderivative steroid is much more active than dexamethasone.

TABLE 8-Inhibition of human airway smooth cell mitogenesis by different concentrations of DEXAMETHASONE and DXN

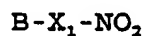
Treatment	Concentration (logM)	³ H-thymidine (CPMx1000)
DEXAMETHASONE	-5	14.1
	-7	15.0
DXN	-5	10.8
	-7	12.6

CONCLUSIONS

As can be observed from results reported above, both activity and safety of the new nitroderivatives are better than those owned by the precursor steroids.

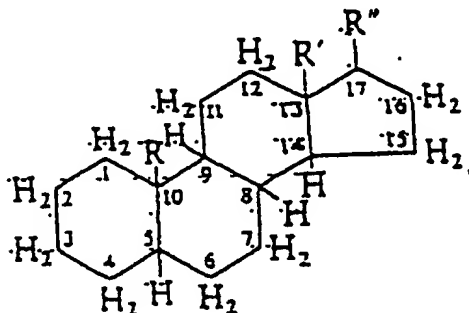
CLAIMS

1. Compounds of the general formula:



or their esters or salts, where:

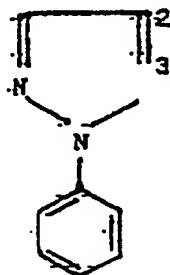
B has the following structure:



where, in place of the hydrogens H in the CH group or two hydrogens H₂ in the CH₂ group shown in the general formula, there may be the following substituents:

at position 1-2: there may be a double bond;

at position 2-3: there may be the following substituent:



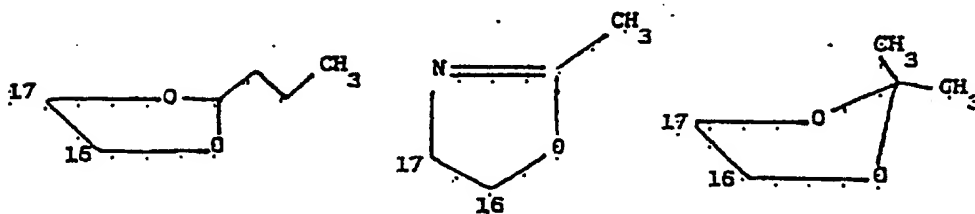
at position 2: there may be Cl, Br;

at position 3: there may be CO, $-O-CH_2-CH_2-Cl$, OH,
 at position 4-5: there may be a double bond;
 at position 5-6: there may be a double bond;
 at position 6: there may be Cl, F, CH_3 , $-CHO$;
 at position 7: there may be Cl;
 at position 9; there may be Cl, F;
 at position 11: there may be OH, CO, Cl;
 at position 16: there may be CH_3 , OH, $=CH_2$
 at position 17: there may be OH, CH_3 , $OCO(O)_{ua}(CH_2)_{va}CH_3$,
 or



where ua is an integer equal to 0 or 1, va is an integer from 0 to 4;

at position 16-17: there may be the following groups



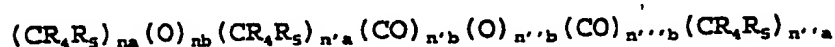
R and R' are equal or different one from the other and may be hydrogen or linear or branched alkyls having

from 1 to 4 carbon atoms, preferably $R = R' = \text{CH}_3$,

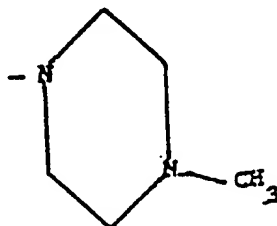
B being a corticosteroid residue;

R'' is $-(\text{CO-L})_t-(\text{X})_{t1}-$

where t and $t1$ are integers equal or different one from the other and equal to 0 or 1, provided that they cannot be both equal to 0 when B contains no -OH groups; the bivalent bridging L is chosen from:



where na , $n'a$, and $n''a$ are equal or different one from the other and are integers from 0 to 6, preferably from 1 to 3, nb , $n'b$, $n''b$ and $n'''b$ are equal or different one from the other and are integers equal to 0 or 1; R_4 and R_5 are equal or different one from the other and are chosen from H, linear or branched alkyl having from 1 to 5 carbon atoms, preferably from 1 to 3; X is equal to X_0 where $X_0 = \text{O}$, NH , NR_{1c} where R_{1c} is a linear or branched alkyl having from 1 to 10 C atoms; or equal to X_2 where X_2 is equal to OH , CH_3 , Cl , $\text{N}(-\text{CH}_2-\text{CH}_3)_2$, SCH_2F , SH ,

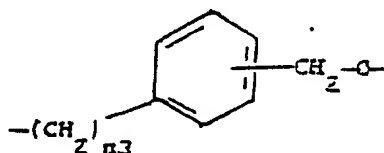


X_1 is a bivalent connecting bridge chosen from:

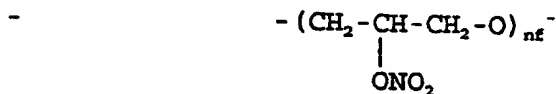
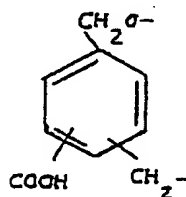
- YO

where Y is a linear or whenever possible branched C_1-C_{20} alkylene, preferably having from 2 to 5 carbon atoms, or an optionally substituted cycloalkylene having from 5 to 7 carbon atoms;

- Y_1 , chosen from



where n_3 is an integer from 0 to 3;



where nf' is an integer from 1 to 6, preferably from 2 to 4;



where $R_{1f} = H, CH_3$, and nf is an integer from 1 to 6, pre-

ferably from 2 to 4.

2. Compounds according to Claim 1, in which precursors include the following, where H_2 , H, R, R', R'' are as defined in the compounds listed below: budesonide, hydrocortisone, alclometasone, algestone, beclomethasone, betamethasone, chloroprednisone, clobetasol, clobetasone, clocortolone, cloprednol, cortisone, corticosterone, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difluprednate, fluzacort, flucloronide, flumetasone, flunisolide, fluocinolone acetonide, fluocinonide, fluocortyn butyl, flucortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, formocortol, falcinonide, halobetasol propionate, halomethasone, halopredone acetate, hydrocortamate, loteprednol etabonate, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, triamcinolone, triamcinolone acetonide, 21-acetoxypregnenolone, cortivazol, amcinonide, fluticasone propionate, mazipredone, tixocortol, triamcinolone hexacetonide, where R'' is as

defined in Claim 1.

3. Compounds or compositions according to Claim 1 to be used as medicaments.
4. Use of the compounds or compositions in Claim 2 for the preparation of medicaments such as corticoids.
5. Use of the compounds or compositions according to Claim 4 for the preparation of medicaments such as anti-arthritic.
6. Use of the compounds or composition according to Claim 4 for the preparation of medicaments such as immunodepressives.
7. Use of the compounds or compositions according to Claim 4 for the preparation of medicaments such as angiostatics.
8. Use of the compounds or compositions according to Claim 4 for the preparation of medicaments such as antiasthmatics.
9. Use of the compounds or compositions according to Claim 4 for dermatology disorders.
10. Use of the compounds or compositions according to Claim 4 for ocular disorders.
11. Use of the compounds or compositions according to Claim 4 for intestinal disorders.

PCT

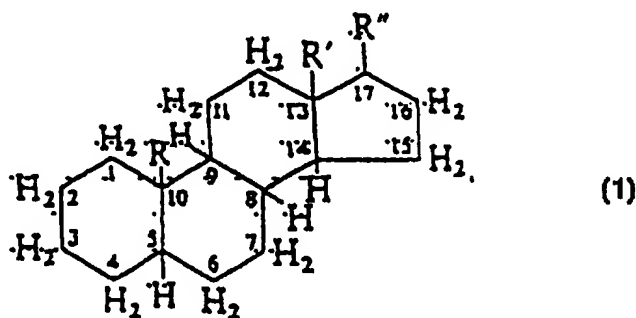
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(54) Title: NITRATE ESTERS OF CORTICOID COMPOUNDS AND PHARMACEUTICAL APPLICATIONS THEREOF



(57) Abstract

Compounds of the general formula $B-X_1-NO_2$ or their esters or salts, where B has structure (1) where there may be substituents in place of the hydrogens H in the CH group or two hydrogens H_2 in the CH_2 group shown in the general formula; R and R' are equal or different one from the other and may be hydrogen or linear or branched alkyls having from 1 to 4 carbon atoms, preferably $R = R' = CH_3$; B being a corticosteroid residue; R'' is $-(CO-L)_t-(X)_{t1}-$ where t and t1 are integers equal or different one from the other and equal to 0 or 1; where L is a bivalent connecting group; X is equal to X_0 where $X_0 = 0, NH, NR_{1C}$ where R_{1C} is a linear or branched alkyl having from 1 to 10 C atoms; or equal to X_2 where X_2 is equal to OH, CH_3 , Cl, $N(CH_2-CH_3)_2$, SCH_2F , SH; X_1 is a bivalent connecting bridge YO where Y is a C_1-C_{20} alkylene.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05426

A. CLASSIFICATION OF SUBJECT MATTER
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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 22 22 491 A (RICHTER GEDEON VEGYESZETI RT) 16 November 1972 see page 4, paragraph 2; examples 2-5,9,10 ---	1-11
Y	DE 16 43 034 A (SCHERING A.G.) 6 May 1971 see page 1 see page 3, paragraph 2; examples 6-12 ---	1-11
Y	BAYUNOVA V I ET AL: "SYNTHESIS AND BIOLOGICAL ACTIVITY OF ALKYL SUCCINATES OF PREDNISOLONE" PHARMACEUTICAL CHEMISTRY JOURNAL, vol. 14, no. 12, 1981, pages 878-880, XP002035558 see the whole document --- -/--	1-11

☒ Further documents are listed in the continuation of box C.

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Watchorn, P

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05426

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HAYASHI H ET AL: "1,4:3,6-DIANHYDROHEXITOL NITRATE DERIVATIVES. II. 1) SYNTHESIS AND ANTIANGINAL ACTIVITY OF ARYL- OR ARYLCARBONYLPIPERAZINE DERIVATIVES 2)" CHEMICAL AND PHARMACEUTICAL BULLETIN, vol. 41, no. 6, June 1993, pages 1100-1110, XP000611905 see page 1100, column 1, paragraph 1 ---	1-11
X	US 3 494 941 A (LEDIG KURT W ET AL) 10 February 1970 see examples 2,4 ---	1,3-11
A	HODOSAN F ET AL: "Nitrate esters of steroid hormones and related compounds. Preparation and biological properties" ARZNEIMITTEL FORSCHUNG. DRUG RESEARCH., vol. 19, no. 4, 1969, AULENDORF DE, pages 684-685, XP002061312 see page 684, column 2 ---	1-11
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 97/05426

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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PCT/EP 97/05426

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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P,X	WO 97 34871 A (GARVEY DAVID S ; LETTS L GORDON (US); NITROMED INC (US); RENFROE H) 25 September 1997 see examples 4,5	1-11
E	WO 97 41144 A (SEARLE & CO ; TJOENG FOE S (US); CURRIE MARK G (US); ZUPEC MARK E () 6 November 1997 see page 6	1-11
E	WO 97 40836 A (SEARLE & CO ; TJOENG FOE S (US); CURRIE MARK G (US); ZUPEC MARK E () 6 November 1997 see claim 1	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/05426

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-11 (in part)

Compounds of the formula B-X1-NO₂, where the nitrate group is attached via or to the 21-position of a pregnane derivative, pharmaceutical compositions thereof and their use in the manufacture of medicaments.

2. Claims: 1-11 (in part)

Compounds of the formula B-X1-NO₂, where the nitrate group is attached to or via the 17-position where the steroid is not a pregnane derivative (i.e. not having a two carbon substituent in position 17.beta.), pharmaceutical compositions thereof and their use in the manufacture of medicaments.

3. Claims: 1-11 (in part)

Compounds of the formula B-X1-NO₂, where the nitrate group is attached via the 11-position, pharmaceutical compositions thereof and their use in the manufacture of medicaments.

4. Claims: 1-11 (in part)

Compounds of the formula B-X1-NO₂, where the nitrate group is attached via the 3-position, pharmaceutical compositions thereof and their use in the manufacture of medicaments.

5. Claims: 1-11 (in part)

Compounds of the formula B-X1-NO₂, where the nitrate group is attached via the 16-position, pharmaceutical compositions thereof and their use in the manufacture of medicaments.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In: .tional Application No

PCT/EP 97/05426

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